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The sexual process of Japanese *Pandorina morum* Bory (Chlorophyta)

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本邦産の *Pandorina morum* Bory (緑藻植物) の有性生殖の過程について

Introduction

Pandorina is one of the common genera of the colonial Volvocales and has a world-wide distribution. Studies on the sexual process under controlled laboratory conditions in this genus have been reported for two species, namely, *Pandorina morum* Bory (Schreiber, 1925; Coleman, 1959) and *Pandorina unicocca* Rayburn & Starr (Rayburn & Starr, 1974). But studies on the sexual process of Japanese materials have not been reported.

In the present study, we observed the sexual process of Japanese strains of *Pandorina morum* in detail under controlled laboratory conditions.

Materials and Methods

Samples Water samples used in this study were collected at Kawai-Dam, Yamanaka-cho, Ishikawa-Prefecture, in August, 1977.

Culture methods Clonal cultures were obtained by the pipette-washing methods (Pringsheim, E. G., 1946) from the samples and were grown in screw cap tubes (18×150 mm) containing 10 ml of growth medium (Table 1). It was modified from M3-medium (Rayburn & Starr, 1974) by adding organic compounds and vitamins. Maintenance of cultures and experimental works were carried out under controlled laboratory conditions. The temperature was kept at about 20°C. Using a bank of cool-white fluorescent lamps, the intensity of illumination was kept at about 4000 lux; 14 hr light-10 hr dark.

Mating methods Mating methods used by Coleman (1959) were applied correspondingly in this experiment. Colonies of two complementary mating types were mixed in a watch glass supported on a glass triangle in petri

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Tab. 1. Growth medium (Modified M3-medium)*.

Component	
KNO_3	101 mg
$MgSO_4 \cdot 7H_2O$	49 mg
$CaCl_2 \cdot 2H_2O$	70 mg
β-Na_2glycerophosphate	153 mg
Glycylglycine	132 mg
Histidine	155 mg
Na acetate$\cdot 3H_2O$	200 mg
Glucose	200 mg
Vitamin B_1	50 μg
Vitamin B_2	5 μg
Vitamin B_6	20 μg
Vitamin B_{12}	0.2 μg
Biotin	10 μg
P IV Metals**	3 ml
Distilled H_2O	997 ml

* pH adjusted to 7.6 with 0.1 N NaOH.

** P IV Metals stock solution, which contains the following (in mg/l) : Na_2EDTA, 1500; $FeCl_3 \cdot 6H_2O$, 194; $MnCl_2 \cdot 4H_2O$, 82; $ZnCl_2$, 10; Na_2MoO_4, 8; $CoCl_2 \cdot 6H_2O$, 4.

dishes. The colonies were mixed by pipetting about 1 ml of each mating type into the watch glass. About 5 ml of distilled water was added to the bottom of the petri dish to minimize water evaporation from the mixture. The heterothallic mating pair of strains observed in this study was obtained by random mixings of five strains from the samples.

Methods of zygote germination Zygotes were planted on the growth medium solidified with 0.6% of agar in petri dishes after about one week from the time of the mating reaction. The agar plate with the zygotes adhering to the surface was put in darkness for about a month. After the dark treatment, the zygotes were transferred from the agar surface to the new liquid medium and placed under the usual illumination.

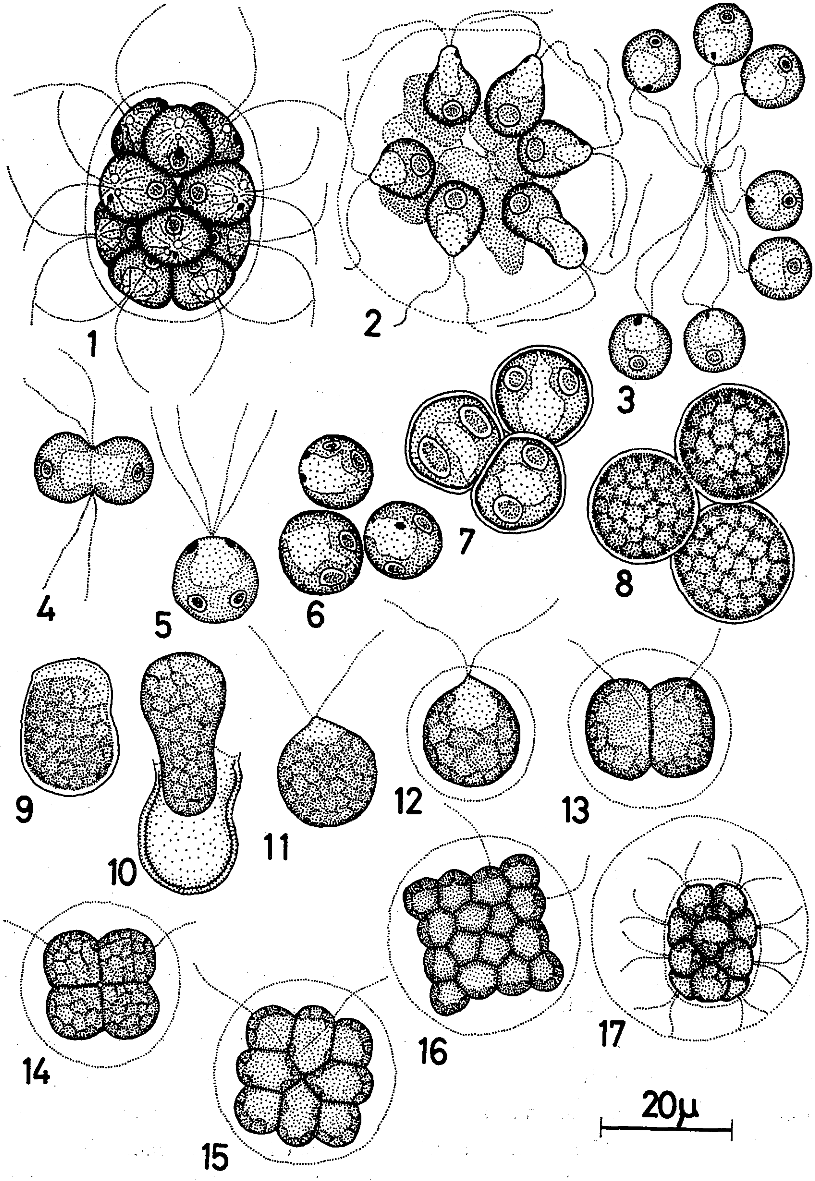
Observation Gelatinous envelopes and flagella were observed by staining with methylene blue and with a phase contrast microscope.

Results

Mating reaction occurs during the earlier light period of the illumination after at least one dark period from the time of the mixing of the colonies. The first step of the mating reaction recognizable is colony clumping, in which several or more number of the colonies combine by mutual entangling of their flagella (C of the plate). In this stage, the shape of the colony does not change at first for about two hours, but afterwards becomes swollen to produce gametes. That is, as the colonial envelope becomes swollen, the constitutive cells become separated from one another and escape from the envelope (Fig. 2). So that all the cells of the colony are released and function as gametes. The gamete is pyriform or ellipsoidal just after the release, but soon rounds up. Except its spherical shape, the gamete has the same form as the vegetative cell: it has a massive cup-shaped chloroplast with one pyrenoid in the bottom and, on the anterior face, one stigma and a pair of flagella of equal length, measured 5-10μm in diameter. This variation of size does not depend upon the mating type but upon the age or physiological conditions of the parental colonies.

After many gametes are released, several or more number of them become associated in a clump with their flagellar tips sticking to one another in the center (gamete clumping) (Fig. 3). During this stage, they draw one another by their flagella. Meanwhile, a pair of gametes, of which sizes are some time same or other not, begins to approach closer and fuses their bodies (Fig. 4). At first, they fuse at the anterior faces opposite to each other, and at last fuse laterally so that the longitudinal axis of each cell orients in the same direction.

As a result, a quadriflagellate zygote is formed (Fig. 5). This motile zygote is spherical in shape, and has two stigmata on its anterior face. It has also two pyrenoids in the bottom of the chloroplast, measured 8-15μm in diameter. After swimming, a large number of zygotes begin to aggregate, settle down with their flagella disintegrated and enter the dormant period (Fig. 6). As a result of this, aplanozygotes are combined by mutual contacts of their surfaces. The mating reaction, mentioned above, from the colony clumping to the beginning of the dormant period, completes during one light period of the illumination. Next step, the zygote loses its stigmata, secretes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a heavy cell wall during the following dark period (Fig. 7) and becomes reddish brown in colour after about one week (Fig. 8). This matured zygote is 10–20μm in diameter. Its internal structure is not seen because of the reddish brown granules in it.

The zygote begins to germ within two days after being transferred from the darkness to usual conditions. The first step of zygote germination is protrusion of one part of the zygote (Fig. 9). As a result of this, the shape of the zygote becomes pyriform or ellipsoidal. Meanwhile, the cell wall of this protrudent region begins to rupture and the reddish brown content (gone cell) escapes, remaining its empty wall (Fig. 10). The gone cell is pyriform or ellipsoidal just after escape, but soon becomes spherical and forms two flagella of equal length then begins to swim slowly (Fig. 11). While they are swimming by these flagella, cell divisions occur.

Before cell division, the gone cell secretes a thin gelatinous envelope around itself (Fig. 12). This envelope is spherical in shape about 20–40μm in diameter, and so thin and transparent that it is difficult to be recognized. In the envelope, successive 3 or 4 divisions occur to form a 8 or 16-celled plakea and a new colony (gone colony) is formed after inversion (Figs. 13–17). The process of this gone colony formation is the same as of the daughter colony formation in the asexual reproduction. The gone colony begins to swim after formation of new flagella. The reddish brown granules recognized in the zygote remain after the gone colony is formed, but vanish gradually to form a green colony as the colony grows. The size of the gone cell depends upon its former zygote. The germination process described above, from the beginning to the gone colony formation, completes during one light period of the illumination.

Besides, during the mating reaction, there are some cells and plakeas which escape from the colonial envelope, settle down on the bottom of the container and conduct colony formation. Such a colony is 4, 8 or 16-celled

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Figs. 1–17. The sexual process of *Pandorina morum* Bory. 1: 16-celled matured colony. 2: Gamete release. 3: Gamete clumping. 4: Fusing gametes. 5: Quadriflagellate zygote. 6: Aplanozygotes before secretion of walls. 7: Green zygotes with heavy walls. 8: Reddish brown zygotes. 9: Germinating zygote. 10: Gone cell escaping from zygote wall. 11: Biflagellate gone cell. 12: Gone cell with gelatinous envlope. 13: 2-celled plakea in gone colony formation. 14: 4-celled plakea in gone colony formation. 15: 8-celled plakea in gone colony formation. 16: 16-celled plakea in gone colony formation. 17: Gone colony in gelatinous envelope.

and often participates in the mating reaction. Such a phenomenon occurs only while they are mating.

Discussion

Our observation on the vegetative phase and the asexual reproduction agreed with the results reported by Coleman (1959). But the mating reaction and the gone colony formation were different.

According to Coleman (1959), colony clumping was not so particular character in the mating reaction. It was reported that 3 to 4 colonies associated with no particular attraction one another. But in the present study, colony clumping was a characteristic phenomenon just before the release of the gametes and several or more number of the colonies participated in a clump (C of the plate). Furthermore, the duration between the mixing of the colonies and the beginning of the mating reaction was not coincident. Coleman reported it as to be 15 to 20 minutes, while in our study, the mating reaction had never been observed unless they passed through at least one dark period of the illumination after the time of the mixing. There may be some possibilities that these differences are resulted from the cultural conditions. But in the studies on another species of the same genus, *Pandorina unicocca* (Rayburn & Starr, 1974; Rayburn, 1974), it was reported that the number of the colonies participating in a clump in colony clumping, and the period from the mixing of the colonies to the beginning of the mating reaction were variable depending upon the pairs of the strains. Based on this fact, the disagreement in *Pandorina morum* between our results and Coleman's (1959) may be caused on the difference of the strains used.

Besides, the gone colony formation observed in the present study was different from that of Coleman (1959). According to her report, a gelatinous envelope surrounding a gone cell was not observed. In the present study, however, a biflagellate gone cell formed a spherical gelatinous envelope around itself (Fig. 12) after escaping from the zygote wall, and cell divisions were performed in it (Figs. 13-17). Such a gelatinous envelope, in which a gone colony is formed, has been reported by several authors in various species; i.e. Pringsheim, N. (1870) and Korschikov (1923) in *Pandorina morum*, Rayburn & Starr (1974) in *Pandorina unicocca* and Harris & Starr (1969) in *Platydorina caudata* Kofoid.

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Explanation of Plate IX

A: Optical section of 16-celled colony showing pyrenoids and stigmata. B: Optical section of 16-celled colony showing colonial envelope and flagella. C: Colony clumping. D: Gamete release. E: Gamete clumping. F: Fusing gametes. G: Quadriflagellate zygote. H: Aggregating zygotes with disintegrated flagella. I: Heavy walled zygotes 2 days after formation, each having two pyrenoids. J: Reddish brown zygotes 10 days after formation. K: Germinating zygote. L: Gone cell escaping from zygote wall. M: Empty walls and zygote. N: Biflagellate gone cell. O: Gone cell with gelatinous envelope. P: 2-celled plakea in gone colony formation. Q: 4-celled plakea in gone colony formation. R: 8-celled plakea in gone colony formation. S: 16-celled plakea in gone colony formation. T: Gone colony in gelatinous envelope.

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本邦産の *Pandorina* 属（緑藻・オオヒゲマワリ目）の有性生殖に関する報告はいまだない。筆者らは石川県の河合ダムから得た *Pandorina morum* Bory の有性生殖の

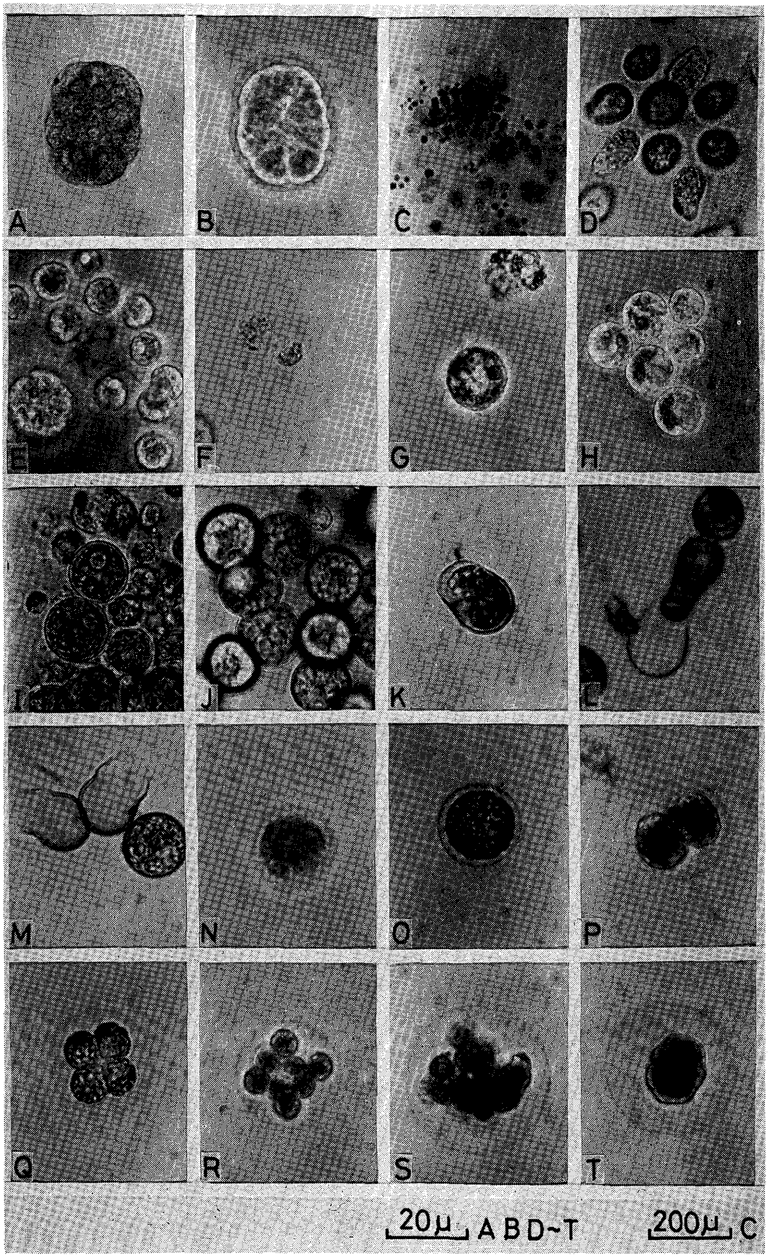
過程を培養条件下で詳細に観察する事ができた。培地はM3-medium (Rayburn & Starr, 1974)に有機物とビタミン類を添加したもので，培養条件は温度約20°C，照度約4000 lux；14 hr light-10 hr darkであった。有性生殖の誘導の方法はColeman (1959)に準じた。有性生殖の過程は基本的にはColeman (1959)の結果と同じであったが，mating reactionとgone colonyの形成に関しては異る結果を得た。

○種類の数の勘定のしかたについて（伊藤　洋）Hirosi ITO: How to count the number of taxa in a certain flora

何々県植物誌，何々山植物目録といったフロラ誌では，総論の部に「本地域の植物は何科何属何種何変種何品種総計何種を数え」などと種類の数を挙げていることが多い。ところが目録に従って数え直してみると，挙がっている数と合わない場合が間々ある。実は間々というより又かというくらい時々行き当たる。科や属の方はほとんど問題ないが，変種や品種を伴った種に問題が多いようである。今簡単のためゼンマイとワラビの二つだけしかないフロラを考えてみよう。学名はそれぞれ*Osmunda japonica*と*Pteridium aquilinum* var. *latiusculum*である。誰が見てもこれは明らかに2種であるが，これが1種1変種だったり2種1変種だったりするのである。恐らくそれはvar.がついていると変種だとばかり思い込んで種を忘れてしまうのか，目録の場合はvar.以下を改行して印刷することが多いため安易に数えてしまうのか，いずれにしてもvar.に釣り込まれる結果ではないかと考えられる。それに，本例には関係ないが*P. aquilinum* var. *aquilinum*のvar.以下を略した場合と取り違えて数えることも起り得るわけである。言い替えると（このような言い方もあまりよくないが）母種と変種の両方がある場合と，変種だけがある場合との混乱である。

上のようなことから数の計上は種までに止めるべしと私は考える。その上ある種では変種や品種がやたらと多く他の種では少ないなどということもあり，また変種や品種の重みというか変異の程度というものも違うので，数だけ数えて多い少ないを誇示したところで意味ないことではなかろうか。なお，和名だけの目録の場合は変種も品種も見かけ上は全く種と同じであるから，合計いくつ（多くの場合合計「何種」と書かれる——合計「何種類」の方がまだましのように思われるが）と数えることは無意味の最たるものであろう。

（東京都文京区[redacted]）



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